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Biochemical Effects of *Telfairia occidentalis* Leaf Extracts against Copper-induced Oxidative Stress and Histopathological Abnormalities

C. U. Ogunka-Nnoka[1], R. Amagbe[1], B. A. Amadi[1] and P. U. Amadi[1*]

[1]Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria.

Authors' contributions

Authors CUO and BAA preconceived and designed the experiment. Author RA managed the analysis of the study, while author PUA performed the data analysis, managed the literature searches and wrote the first draft of the manuscript. All authors approved the final manuscript.

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Original Research Article

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ABSTRACT

This study was carried out to investigate the protective effect of oral administration of *T. occidentalis* against copper induced oxidative stress. Forty two adult male Wistar rats were divided equally into six groups. Group 1 were orally gavaged with standard animal feed only, group 2-6, in addition to normal feed received oral treatment of 0.3 mg/kg b.w copper daily, group 3 and 4 received 250 mg/kg b.w and 500 mg/kg b.w aqueous leaf extract of *T. occidentalis* respectively, while group 5 and 6 were treated with 250 mg/kg and 500 mg/kg b.w methanol extract of *T. occidentalis*. The oral administration of 500 mg/kg b.w proved most effective in the restoration of the antioxidant enzyme status, while the hepatoprotective activity of the extracts was completely effective on the AST levels, total bilirubin, and serum albumin levels. The results for renal function markers show that only 500 mg/kg b.w methanol extract completely restored the urea and potassium levels. This indicates that *T. occidentalis* could ameliorate the antioxidant status in copper-induced oxidative stress.

**Corresponding author: E-mail: peter_amadi@uniport.edu.ng, amadi.peter.u@gmail.com;*

Keywords: Methanol extract; oxidative stress; hepatotoprotective activity; antioxidant enzyme; renal function markers.

1. INTRODUCTION

In humans, the involvement of oxidative stress in the development of certain diseases and/or exacerbation of their symptoms has been well documented. They are initiated by free radicals like nitric oxide, hydrogen peroxide, superoxide anions, and hydroxyl radicals. Free radicals and reactive oxygen species have been reported to cause the disruption of the integrity of biological systems, thereby contributing to the pathogenesis of some diseases like cancer, diabetes, arthritis, and atherosclerosis [1]. The damaging effects of these free radicals are however cushioned by some endogenous antioxidants like glutathione reductase, catalase, tocopherol, superoxide dismutase, ascorbic acid etc. That notwithstanding, these endogenous antioxidants have been found to be insufficient in the complete prevention of the effects of free radicals. In such events where the activities of these antioxidants are inadequate, the administration of exogenous antioxidants becomes imperative. Unfortunately, many reports have argued that the administration of synthetic exogenous antioxidants evoke numerous side effects, whereas natural plant sources of antioxidants exhibit very strong antioxidant effects due to the presence of components such as phenols, flavonoids, flavonols, proanthocyanins, lycopene etc [2]. In most African countries like Nigeria, plants play several roles in traditional medicine due to the abundance of different natural products. The ingestion of these plant sources of natural products have been identified as the hallmark of disease prevention [3,4,5]. Nowadays, researchers tailor their studies towards the potential health effects of the phytochemical constituent of these foods and vegetables [6]. Plants are good sources of antioxidants, and *Telfairia occidentalis* have been one of the most investigated due to its antioxidant properties and free radical scavenging potentials [7,8,9]. *Telfairia occidentalis* is a member of the cucurbitaceae family and is indigenous to South East Nigeria. Common names for the plant include Fluted gourd, Fluted pumpkin, and Ugu. The fluted gourd grows in many nations of West Africa but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines. The leaves of *T. occidentalis* are attributed to be nutritious and medicinal, and as a result, are consumed in different parts of the country. Several extracts of the leaf have been reported to elevate the antioxidant enzyme levels both in vivo and in vitro, and scavenge, prevent or suppress the production of free radicals [7,9,10]. Studies have specifically attributed the antioxidant effects of *T. occidentalis* leaf to its high ascorbic acid and phenol content [11]. Hamza et al. [12] have argued that the extracts of *T. occidentalis* gotten from the usage of different enzyme solvents offered protective and chemopreventive effects on oxidative stress-induced organs like brain, liver, and kidney. *T. occidentalis* is known to contain high superoxide and hydroxyl radical scavenging potentials, high total antioxidant and phenol content, and lipid peroxidation inhibition potentials [12]. In addition, Nwanna and Oboh, [13] have reported the hepatoprotective characteristics of the polyphenol contents of *T. occidentalis* leaves on acetaminophen-induced liver damage. Therefore, it is possible that by consuming the leaves of *T. occidentalis*, the provision of sufficient antioxidants that can prevent or cushion the effects of oxidants and promote the general well being of animals, is assured. It is to this end that this study was carried out to evaluate the modulatory effect of aqueous and methanol leaf extracts of *T. occidentalis* against copper-induced oxidative stress and histopathological abnormalities.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Fresh pumpkin leaves were bought from Choba Market in Rivers State Nigeria, authentication of the leaves was carried out in the Department of Plant Science and Biotechnology, University of Port Harcourt Choba, Rivers State, Nigeria, and was sun dried for 4 days before being ground into powdery form using an electronic grinding machine (Dade:DFT-50).

2.2 Preparation of Aqueous Extract

A 300 g of the powdered *Telfairia occidentalis* was measured and soaked in 600ml of distilled water for 48 hours and afterwards sieved using a muslin cloth before being filtered with Whatmann paper size 1. The filtrate was thus concentrated using a Rotory Evaporator at 40ºC.

2.3 Preparation of Methanolic Extract

The powdered *Telfairia occidentalis* (300 g) was measured and soaked in 600 ml of 75% methanol for 48 hours and afterwards sieved using a muslin cloth before filtering with Whatmann paper size 1. The filtrate was thus concentrated using Rotory Evaporator at 40ºC.

2.4 Chemicals and Reagents

They include the following: Copper sulphate, Chloroform (BDH chemicals Ltd), methanol 95% (SIGMA), Formalin 10%, Biochemical reagent kits (MINDRAY) and Finisher feed (Top Feed Ltd).

2.5 Experimental Animal

Forty two (42) male Wister rats weighing 170-200 g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. The animals were acclimatized to house conditions for seven days (7), and provided with normal feed (Top feed grower's mash) and clean water *ad libitum*. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care [14].

2.6 Experimental Design

Animals were weighed and randomly assigned into six equal groups, namely-

Group 1: Received only normal feed and water

Group 2: Received intraperitoneal injection of 0.3 mg/kg body weight (b.w) of copper sulphate once daily to induce oxidative stress, with no further treatment administered.

Group 3: Received intraperitoneal injection of 0.3 mg/kg body weight of copper sulphate once daily, and was treated with 250 mg/kg body weight of the aqueous extract of *Telfairia occidentalis* leaves.

Group 4: Received intraperitoneal injection of 0.3 mg/kg body weight of copper sulphate once daily, and was treated with 500 mg/kg body weight of the aqueous extract of *Telfairia occidentalis* leaves.

Group 5: Received intraperitoneal injection of 0.3 mg/kg body weight of copper sulphate once daily, and was treated with 250 mg/kg body weight of the methanolic extract of *Telfairia occidentalis* leaves.

Group 6: Received intraperitoneal injection of 0.3 mg/kg body weight of copper sulphate once daily, and was treated with 500 mg/kg body weight of the methanolic extract of *Telfairia occidentalis* leaves.

All groups were fed with the same animal feed and water *ad libitum*.

After 28 days, the animals to be sacrificed were first subjected to inhalational anesthesia with chloroform followed by cervical dislocation. Each of the experimental animals were placed on a dissecting slab, and cut along the thorax down the abdominal region. Blood was collected via cardiac puncture and dispensed into the Heparin bottle for biochemical assays.

2.7 Biochemical Parameters

2.7.1 Determination of oxidative stress parameters

2.7.1.1 Determination of Malondialdehyde [15]

Normal saline (0.5 ml) was pipetted into a test tube containing 0.5 ml of the serum sample. About 2 ml of thiobarituric acid (TBA)/trichloroacetic acid (TCA) mixture was added, allowed to boil for 1 hour, cooled to room temperature, and centrifuged at 4000 rpm for 5 min. The clear supernatant was read at 535 nm.

2.7.1.2 Determination of Catalase activity [16]

Distilled water (2.5 ml) was pipetted into test tube containing 0.5 ml H_2O_2, and about 40 µl sample was added and mixed thoroughly. Rate of decomposition of hydrogen peroxide was read at 240 nm at 30 sec interval for 5 mins.

2.7.1.3 Estimation of Superoxide Dismutase (SOD) activity [17]

Sample extract (20 ml) and 2.5 ml of 0.05 M carbonate buffer (pH 10.2) were mixed together and equilibrated in the spectrophotometer. In addition, 0.3 ml of 0.3 mM freshly prepared adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 3 mins.

2.7.1.4 Estimation of Glutathione (GSH) [18]

To a test tube containing 0.5ml of the sample was added 0.5 ml (50%) of TCA and the solution was mixed and centrifuged at 2.0 x 10^3 rpm. Then, 1 ml of the supernatant was mixed with 2ml of 0.01 m DTBN reagent (Ellman's reagent) and kept away from direct light for 15 to 20 minutes. The absorbance at 412 nm was recorded. Then, standard glutathione was added to a mixture of 1.5ml phosphate buffer and 2 ml of DTBN, and absorbance was read at 412 nm after 15 minutes. The concentrations of glutathione (µg/ml) were traced from the standard curve for glutathione.

2.7.1.5 Determination of Glutathione Peroxidase (GPx) [19]

Glutathione peroxidase (GSH-px) activity in the sample was measured using Randox GSH-px kit according to the method of Paglia and Valentine, [19].

2.8 Liver Function Test

2.8.1 Liver enzymes

Concentrations of AST, ALT, and ALP were obtained by kinetic methods with kits from Mindray test kits (Mindray Medical International Limited, China) using a double-beam spectrophotometer. Other reagents used were of analytical grade.

2.8.2 Renal function test

2.8.1.1 Determination of creatinine

A Modified method according to Bartels and Bolmer [20] was used to determine the level of Creatinine in the samples. Mindray test kits (Mindray Medical International Limited, China) were used for the analysis.

2.8.2.2 Determination of urea

Urease-glutamate Dehydrogenase -UV method according to Berthelot's method [21] was used to determine the level of Urea in the samples. Mindray test kits (Mindray Medical International Limited, China was used for the analysis.

2.8.2.3 Determination of Serum Abumin (ALB) level

Bromcresol green (BCG) method was used to determine the level of Plasma Albumin in the samples according to the method of Doumas et al. [22].

2.8.2.4 Determination of Serum Total Protein (TP)

Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen, [23] and Burtis [24].

2.8.2.5 Determination of Total Bilirubin (T-BIL) concentration

Jendrassik-Grof method [25] of Mindray test kit was used to determine the level of Total Bilirubin in the samples.

2.8.2.6 Determination of serum electrolyte

Na^+ and K^+ were determined by flame photometery using Jenway P7 Flame photometer. Chloride ion activities of serum enzymes were determined using diagnostic kits (Quinica Clinica Applicada S.A Spain) following Reitman and Frankel [26] method.

2.8.2.7 Histological examination

The liver and kidneys of a rat from each group after being removed from surrounding tissues were fixed in 10% formal saline and after 72 h the organs were dehydrated in graded alcohol (20%, 30%, 50%, 70%, 95%), 5 min each, cleared in xylene, and embedded in paraffin. The resulting blocks were completely sectioned and randomized. The selected sections were stained in haemotoxylin and eosin and the slides were then examined at magnification of ×400 under optical microscope.

2.9 Statistical Analysis

All data were subjected to statistical analysis. Values are reported as Mean ± Standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 20. The results were considered significant at p-values of less than 0.05 ($p<0.05$).

3. RESULTS AND DISCUSSION

The results of administration of *T. occidentalis* extracts on MDA levels of Wistar rats with copper-induced oxidative stress (Fig. 1) shows a significant derangement of antioxidant system on

exposure to copper. Response related increase in the levels of MDA on exposure to inducers of oxidative stress is well documented. Ali and Atalla, [27] reported a significant increase in MDA levels after administration of Dimpylate. Monosodium glutamate, paracetamol, and carbon tetrachloride when used as oxidative stress inducers, all significantly increased the MDA levels which were in agreement with this present study [28,29,30]. Dervis et al. [31] similarly reported an increase in MDA levels after exposure to copper. MDA is also an indicator for lipid peroxidation [29]. The results of this present study showed that the significant decrease in the levels of MDA on treatment with aqueous extract of *T. occidentalis* was not concentration dependent. Further, methanol extracts at both concentration used completely restored the activity of MDA when compared to the control group. This complete restorative activity of *T. occidentalis* on MDA was on par with *Matricaria chamomilla* extracts [27], *Coeletura aegyptiaca* [29], and cinnamon extracts [28]. The increase in MDA levels was for the augmentation of oxidative stress in tissues but the significant ameliorative potentials of both aqueous and methanol extracts of *T. occidentalis* alleviated this increase.

A significant decrease in the activity of SOD was observed on treatment with copper (Fig. 2). The administration of both 250 mg/kg b.w and 500 mg/kg b.w aqueous extracts of *T. occidentalis* was found ineffective in restoring the activity of SOD. Treatment with 250 mg/kg b.w methanol leaf extract of *T. occidentalis* significantly increased the concentration of SOD which increased significantly on further increment to 500mg/kg b.w administered methanol leaf extract. However, the restoration of the activity of SOD on administration of 500mg/kg b.w methanol leaf extract was not absolute, as shown in Fig. 2. Ethanol extracts of *Mangifera indica* stem barks offered more SOD restorative potentials [30] than methanol extracts of *T. occidentalis* in this study. Although in line with this present study, *Tinospora cordifolia*, and *Emblica officinalis* were also unable to completely restore the activity of SOD against Cd-induced oxidative stress and alcohol-induced oxidative stress respectively [32,33]. Superoxide dismutase catalyzes the dismutation

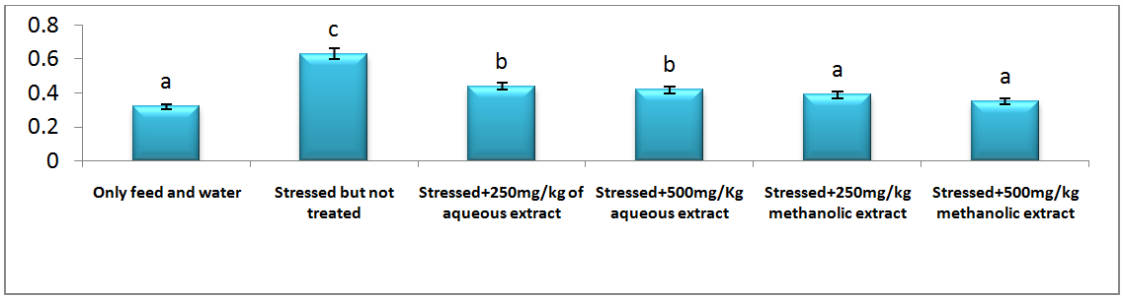

Fig. 1. Effect of *T. occidentalis* extracts on malondialdehyde levels (umol/L) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-c) are not significantly (p<0.05) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

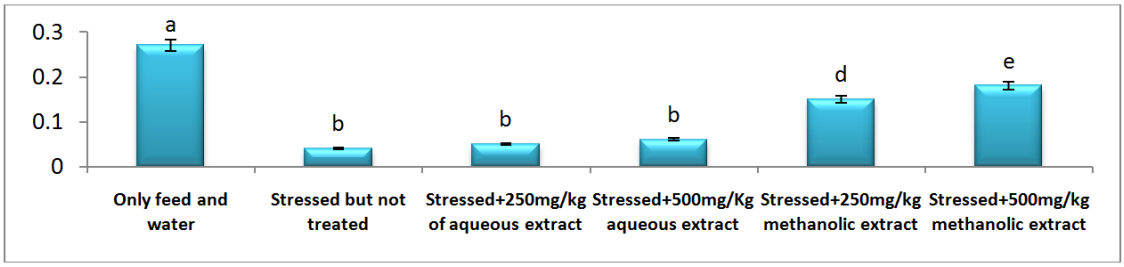

Fig. 2. Effect of *T. occidentalis* extracts on superoxide dismutase levels (umol/L) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-e) are not significantly (p<0.05) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

of superoxide anion to hydrogen peroxide, for further detoxification to water by catalase [34]. The decrease in activity of SOD after treatment with copper in this present study implies that the synthesized antioxidant enzyme has been suppressed. This is in line with the findings of Omotayo et al. [30].

The effect of *T. occidentalis* extracts on glutathione levels (ug/L) of Wistar rats with copper-induced oxidative stress was shown in Fig. 3. Copper-induced oxidative stress significantly reduced the glutathione levels which significantly increased after the administration of 500 mg/kg b.w aqueous and methanol leaf extract. Dose level of 200 mg/kg b.w extract of *Baphia nitida* was able to significantly restore the glutathione levels after diazepam induced oxidative stress in rats [35]. Khan et al. [36] reported that 200mg/kg b.w various fractions of *L. procumbens* suppressed the toxic effects of $KBrO_3$-induced oxidative stress, while reversing the activity of the enzymes near to the control group. In line with the findings of this present study, Khan et al. [36] and Sahreen et al. [37] have also reported similar findings with other plant extracts. Fuentealba et al. [38] posited that glutathione is activated in advanced exposure to copper. This implies that the toxicity of copper could strongly depend on the significant reduction in the intracellular levels of glutathione.

The GPx levels of Wistar rats with copper-induced oxidative stress after treatment with aqueous and methanol extracts of *T. occidentalis* was shown in Fig. 4. The significant decrease in GPx levels after treatment with copper was slightly ameliorated by the administration of 250 and 500 mg/kg b.w aqueous leaf extract of *T. occidentalis*, and 250 mg/kg b.w methanol leaf extract of *T. occidentalis*. Treatment with 500 mg/kg b.w methanol leaf extract of *T. occidentalis*, proved most effective (Fig. 4) but was still significantly lower than the GPx levels of the control group. GPx is regarded as one of the key enzymes for the detoxification of reactive oxygen species. *Matricaria chamomilla* did not completely restore the activity of GPx [27], but grape seed proanthocyanidine extracts did completely restore the Gpx activities against oxidative stress induced by cisplatin in rats. The restorative potentials of *Tinospora cordifolia* extract on Cd-induced oxidative stress in rats, compared significantly to the control group [33].

Catalase plays a central role in the decomposition of hydrogen peroxide to water and oxygen [39]. From the statistical point of view, treatment with 250mg/kg b.w aqueous leaf extract of *T. occidentalis* was ineffective, while no significant difference was recorded for the catalase activity after treatment with 500mg/kg b.w aqueous leaf extract and 250mg/kg b.w methanol extract of *T. occidentalis*. Total restoration of the activity of catalase was achieved on administration of 500mg/kg b.w methanol extract as shown in Fig. 5. Omoregie and Osagie, [40] have reported that the supplementation of protein deficient diet with methanol extracts of *A. hybridus, J. tajorensis, G. Africana, T. triangulare,* all enhanced the activity of catalase. On a similar note, 200mg/kg b.w of *B. nitida* ethanol extracts similarly restored the activities of catalase.

The effect of *T. occidentalis* aqueous and methanolic extracts on liver markers (U/L) of Wistar rats with copper-induced oxidative stress

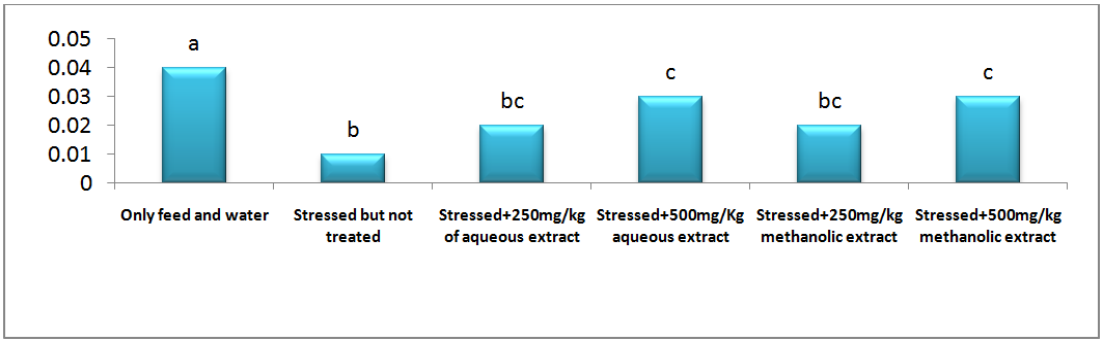

Fig. 3. Effect of *T. occidentalis* extracts on glutathione levels (ug/L) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-c) are not significantly ($p<0.05$) different*

**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

are shown in Fig. 6. It is well documented that oxidants generally cause harm to the liver tissues. AST and ALT are cytosolic enzymes and are important biomarkers of liver damage. In this present study, the administration of copper significantly elevated all liver enzyme markers. The elevation in the plasma levels of transaminases on treatment with copper indicates impairment of liver functions. Transaminases are regarded as the most sensitive biomarkers with direct implication in the extent of cellular distortion and toxicity because their cytoplasmic location enables their release into the circulation after cellular damage [41]. Alterations in AST and ALT are reported in hepatic disease and in myocardial infarction [42]. In this present study, alterations in the AST levels were shown to be completely restored only by 250 mg/kg b.w of the aqueous leaf extract of *T. occidentalis*. However, significant differences were recorded for other treatment groups, but none were comparable to the control group. Treatment using 250 mg/kg b.w of aqueous leaf extract did not significantly attenuate the toxicity posed by copper on ALT (Fig. 6). However, treatment using 500 mg/kg b.w aqueous extract, 250 mg/kg b.w of methanol leaf extract, and 500 mg/kg b.w of methanol leaf extract showed significant hepatoprotective potentials. The results have thus shown that hepatoprotective activities of these extracts is considered to be dose dependent especially for AST and ALT while ALP were unaffected by dose. The findings of Omotayo et al. [30] and Malami et al. [43] are in line with the findings of this present study showing a decrease in activity of ALT, AST, ALP in rats with CCl_4 induced hepatotoxicity and treated with aqueous stem bark extract of *M. indica*.

The restoration of total protein levels, to normal state after treatment with copper and extracts, proved to be concentration dependent. The total protein levels after treatment with 500mg/kg b.w aqueous extract were comparable to that of the control group (Fig. 7). The functional status of the liver is reflected by total protein [44] because the liver is enriched with machineries for the synthesis of serum proteins excluding γ-globulins, hence, liver damage is characterized by hypoproteinemia which can affect the whole physiological status of animals [45,46]. In this present study, a significant reduction in total protein observed in exclusively copper treated groups in relation to control group indicates hypoproteinemia, while the significant increase in the aqueous and methanol treated groups suggests increased protein synthesis. Serum total proteins have also being reported to have significantly decreased in Dimpylate intoxicated rats as compared to the group not treated with the oxidant [27]. On another note, Yousef *et al.* [56] found that the plasma total protein significantly decreased after administration of Cisplatin but significantly increased in comparison with the control group after treatment with grape seed proanthocyanidin extract.

Fig. 8 shows the bilirubin levels (Umol/L) of *T. occidentalis* extract-treated Wistar rats with copper-induced oxidative stress. Oxidative stress have been shown to increase the bilirubin levels, as indicated

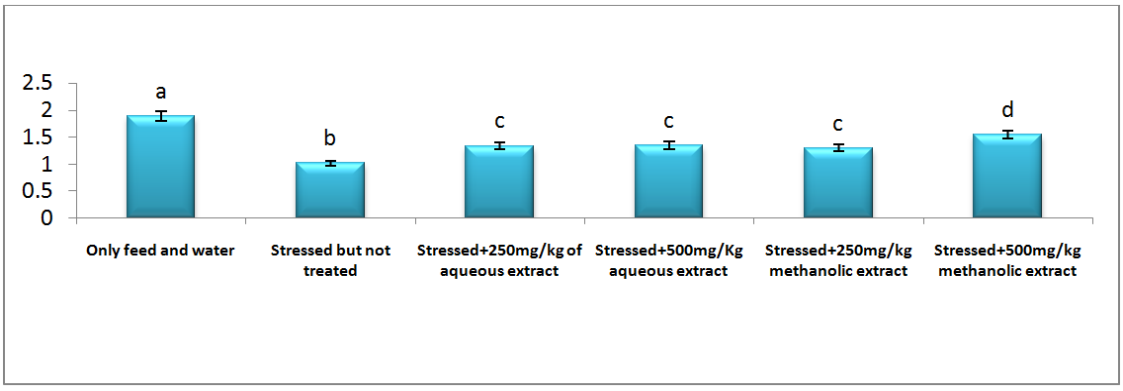

Fig. 4. Effect of *T. occidentalis* extracts on glutathione peroxidase (GPx) levels (ug/L) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-d) are not significantly ($p<0.05$) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

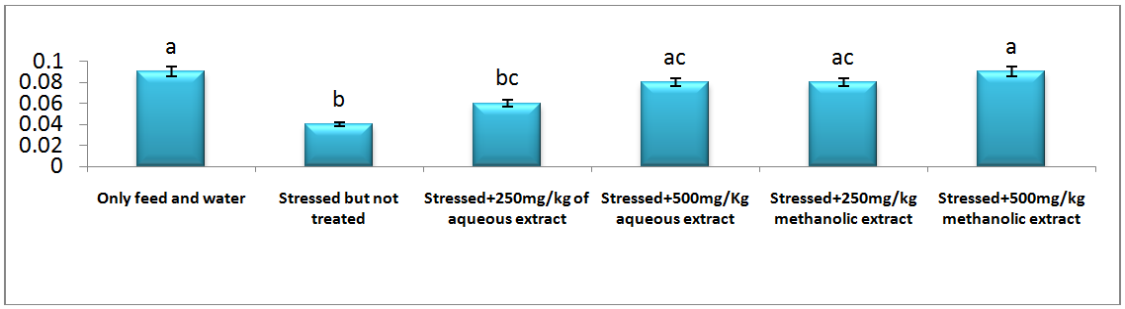

Fig. 5. Effect of *T. occidentalis* extracts on catalase levels (u/ml) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-c) are not significantly (p<0.05) different*
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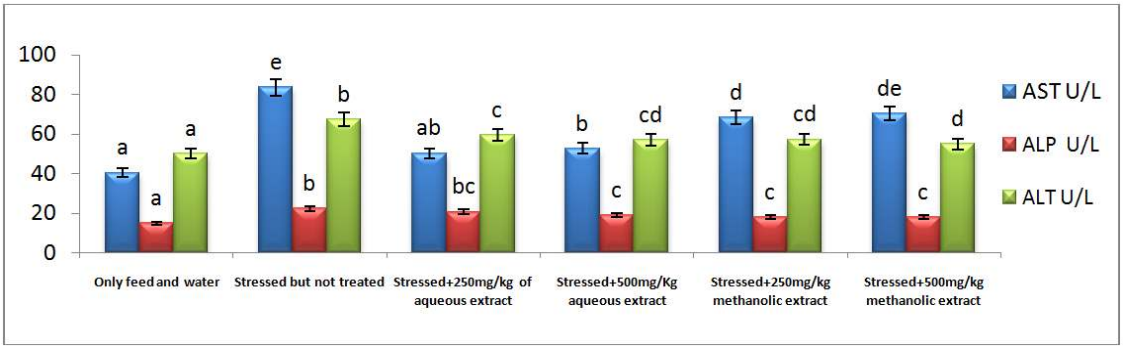

Fig. 6. Effect of *T. occidentalis* aqueous and methanolic extracts on liver markers (U/L) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-e) separately for each enzyme, are not significantly (p<0.05) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

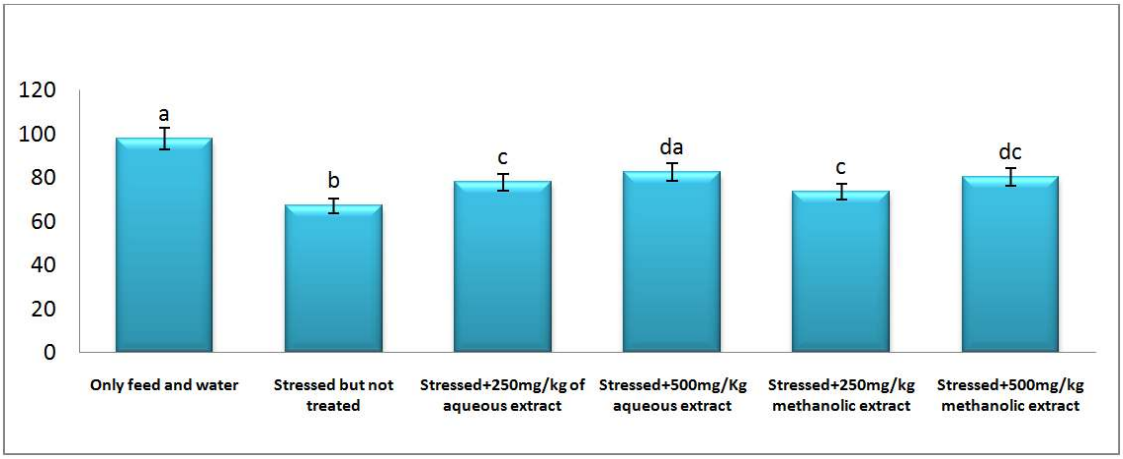

Fig. 7. Total protein levels (g/L) of *T. occidentalis* extract- treated-Wistar-rats with copper-induced oxidative stress

**Columns bearing similar letters (a-d) are not significantly (p<0.05) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

in Fig. 8 from the treatment group subjected only to intraperitoneal administration of copper. According to Sanjiv, [47] the level of bilirubin, which is a major breakdown product of haemoglobin, increases on account of liver damage which manifests as skin discolouration. The reports of Anosike et al. [48], in agreement with the findings of this study, showed that oxidative stress induced by CCl_4 elevated the total bilirubin levels. A similar report has been found for ethanol induced oxidative stress on bilirubin levels [49]. The results of this present study show that the restoration of total bilirubin by methanol extracts of *T. occidentalis* occurred in a dose dependent manner up to 500 mg/kg b.w. Anosike et al. [48] using ethanol extract of *Pyrenacantha staudti* reported the complete restoration of total bilirubin levels at 400 mg/kg b.w. Amelioration of copper induced oxidative stress depletion of total bilirubin by 500 mg/kg b.w methanol leaf extract of *T. occidentalis* further show its protective effect against copper induced liver toxicity. The extract may have exerted its hepatoprotective effects by enhancing bilirubin uptake by the liver and subsequent secretion into the bile ducts.

The results for the albumin levels (g/L) of *T. occidentalis* extract-treated Wistar rats with copper-induced oxidative stress are shown in Fig. 9. The significant reduction in albumin levels on treatment with copper is in line with other similar studies. Ojelabi et al. [50] reported a derangement in albumin activity during cadmium mediated oxidative stress. Yakubu et al. [51] also reported a significant reduction in albumin levels on intubation of acetaminophen as an oxidant. In this present result, the LSD test carried out showed that only 500 mg/kg b.w aqueous leaf extract and 500 mg/kg b.w methanol extract completely restored the albumin activities. Similarly, Ebenyi et al. [52] revealed that the extract of *A. sativum* produced a significant increase ($p < 0.05$) in the albumin level in rats

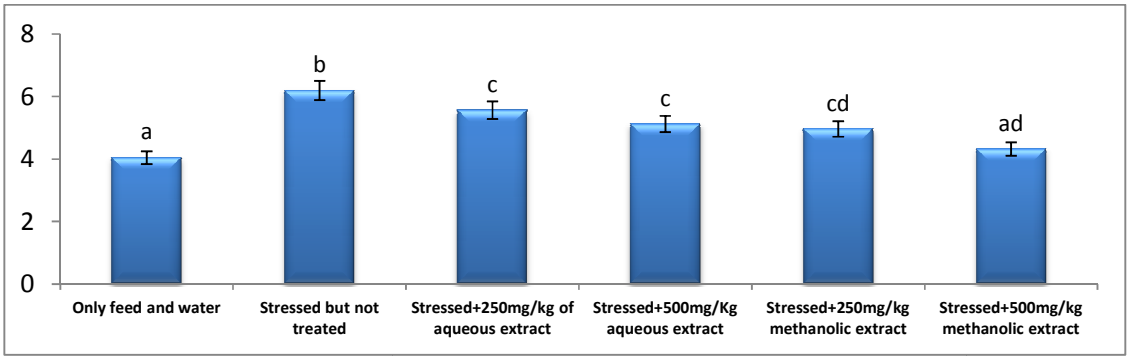

Fig. 8. Bilirubin levels (µmol/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-d) are not significantly (p<0.05) different*
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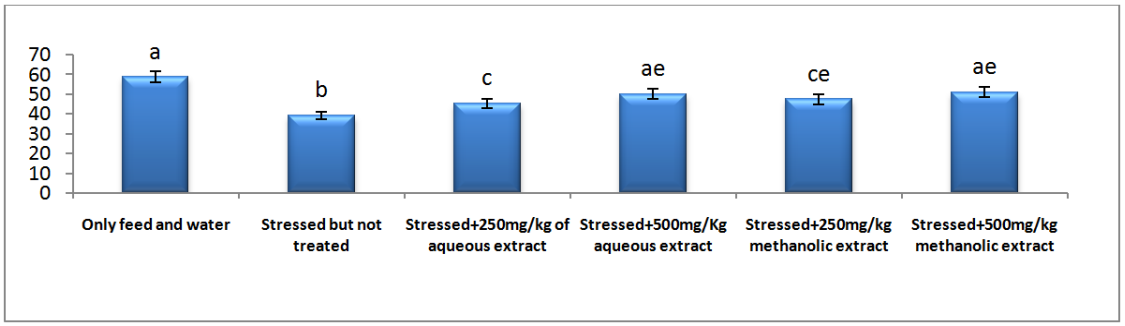

Fig. 9. Albumin levels (g/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-e) are not significantly (p<0.05) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

with paracetamol-induced oxidative stress, in a dose dependent manner. This was in consonance with the findings of this present study. Albumin is important in reducing the bioavailability and toxicity of many substances, by binding them. This binding potential of albumin to xenobiotics is reduced in occurrence of hypoalbuminaemia which causes the blockage of the binding sites by various metabolites. Although this hepatoprotective activity of albumin cannot be related to any specific phytochemical constituent of the extracts, some of the constituent phytochemicals like flavonoids and phenols are known to posses antioxidant properties.

The treatment with *T. occidentalis* aqueous and methanol leaf extract on copper induced oxidative stress on electrolyte markers were shown in Fig. 10. The results showed a significant increase in urea levels from 4.73 mmol/l to 8.77 mmol/l after inducing oxidative stress. A similar oxidative stress induced increase was obtained for the creatinine levels on treatment with copper. Administration of both 250 mg/kg b.w and 500 mg/kg b.w aqueous leaf extract were unable to restore the activities of urea and creatinine, though caused a significant reduction in their levels when compared to the exclusively copper treated group, with only creatinine showing a dose dependent amelioration on aqueous leaf extract treatment. In contrast, according to Nwangwa, [53] *Carica papaya* seed extracts were able to completely reverse the CCl_4 induced renal toxicity in rats monitored through their urea and creatinine levels. The results of treatment with methanol leaf extract on creatinine as shown in Fig. 10 of this present study indicated the inability of the concentrations used to completely restore the creatinine levels, but significantly reduced the serum urea levels comparable to the control group. Oxidative stress conversely reduced the serum potassium, sodium, and chloride levels as shown in Fig. 10. However, on administration of 500 mg/kg b.w of both extracts, the potassium levels were completely restored, while none of the concentrations used in this present study were sufficient to completely restore the serum concentration of Na^+ and Cl^- after copper induced oxidative stress. Ali et al. [54] also noted a significant alteration in selected serum electrolytes in the rats exposed to ethanol induced oxidative stress. The findings of this present study were similar to the report of Grover et al. [55] on the effect of *Piper guineense*, and *G. latifolium* during oxidative stress.

The histological section of the kidney tissue of the rat in Plate A, shows normal renal tubules while the histological section of the kidney of the rat orally treated with 0.3 mg/kg body weight of copper sulphate but not treated with the extracts, shows inflammation in renal tubules (Plate B). Histological section of the kidney of the rats induced with 0.3 mg/kg body weight of Cu and treated with aqueous extract of 250 mg/kg body weight showed ameliorations (Plate C) and increment to 500mg/kg body weight of *Telfairia occidentalis* showed normal renal tubules (Plate D). Treatment with methanolic extract of 250 mg/kg body weight of *Telfairia occidentalis* coupled with copper (0.3 mg/kg) showed normal renal tubules (Plate E) and an increase to 500 mg/kg body weight of *Telfairia occidentalis* showed normal renal tubules (Plate F).

Histological study of the liver tissue of the rat in the group 1 (Plate G) showed normal hepatocytes and portal vein and on

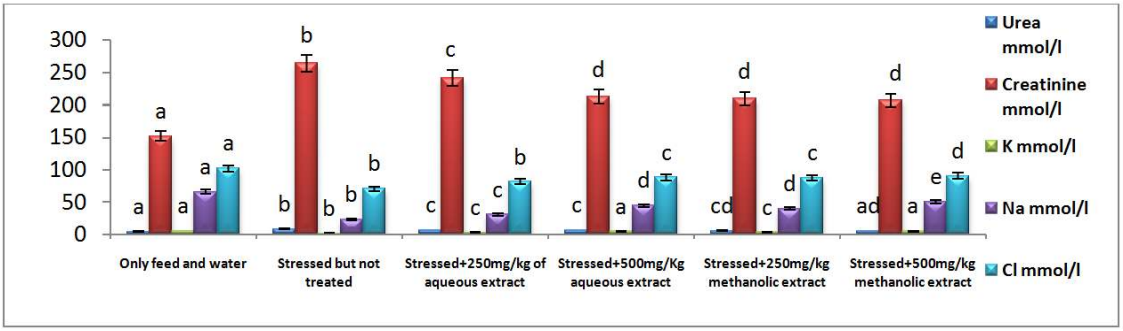

Fig. 10. Effect of *T. occidentalis* extracts on electrolyte markers (mmol/l) of Wistar rats with copper-induced oxidative stress

**Columns bearing similar letters (a-e) separately for each marker, are not significantly ($p<0.05$) different*
**Stressed implies received intraperitoneally, 0.3 mg/kg body weight of copper sulphate once daily*

administration of 0.3 mg/kg body weight of copper sulphate without any extract treatments, showed destruction of liver cells as seen in Plate H. The administration of Cu (0.3 mg/kg) body weight with 250 mg aqueous *T. occidentalis* extract in Plate I on histological section of liver of the rats shows recovering liver tissues central vein and cords of hepatocytes. Histological section of the liver of the rats induced with Cu (0.3 mg/kg) body weight and treated with aqueous extract of 500 mg/kg body weight of *Telfairia occidentalis* shows normal liver tissues central vein and cords of hepatocytes (Plate J) while histological section of the liver of the rats induced with Cu (0.3 mg/kg) body weight and treated with methanolic extract of 250 mg/kg body weight of *Telfairia occidentalis* (Plate K) showed restoration of liver tissues central vein and cords of hepatocytes while histological section of the liver of the rats induced with Cu (0.3 mg/kg) body weight and treated with methanolic extract of 500 mg/kg body weight of *Telfairia occidentalis* (Plate L) showed restoration of liver tissues central vein and cords of hepatocytes.

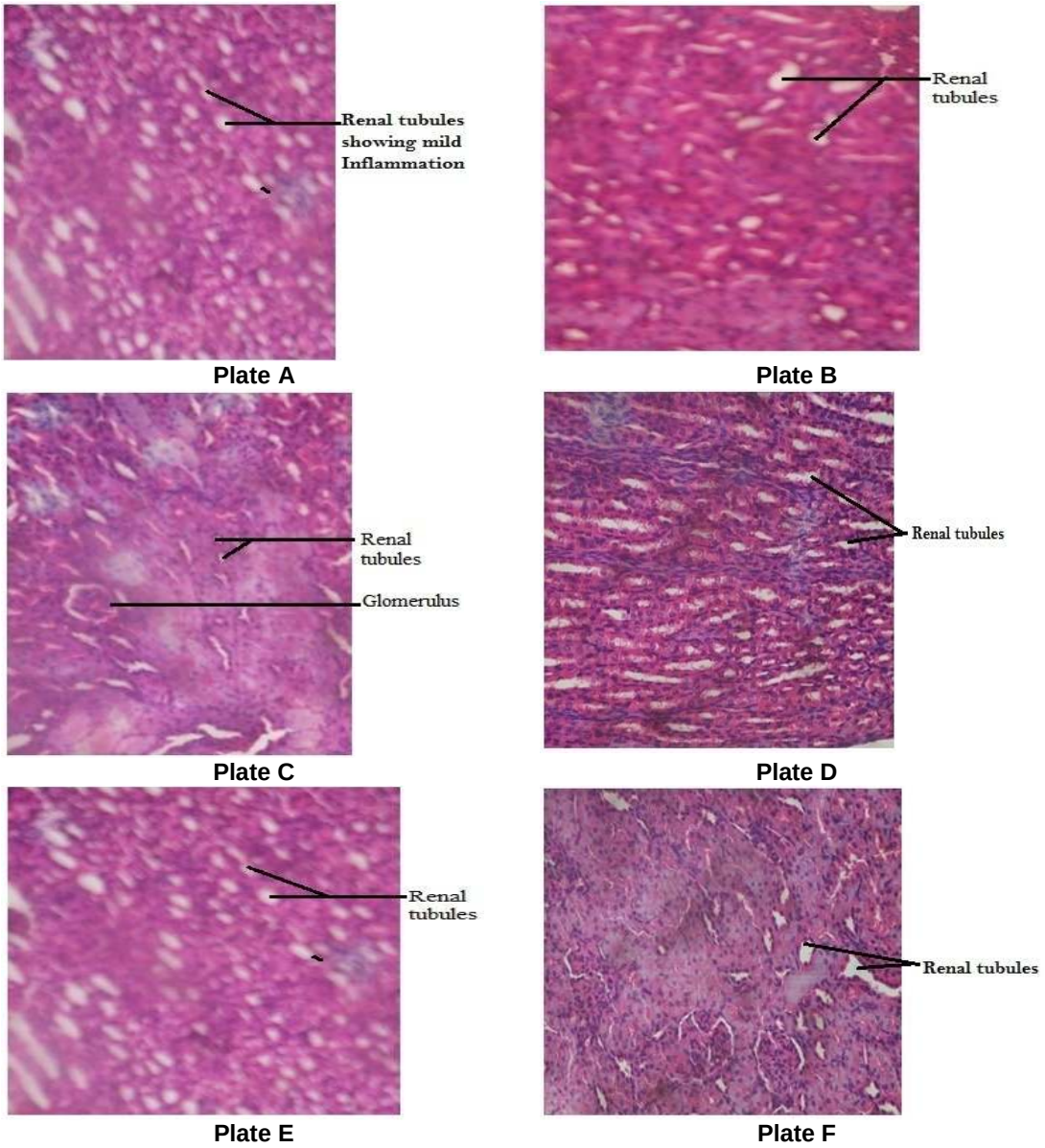

Plate A-F. Histopathology examination of kidney

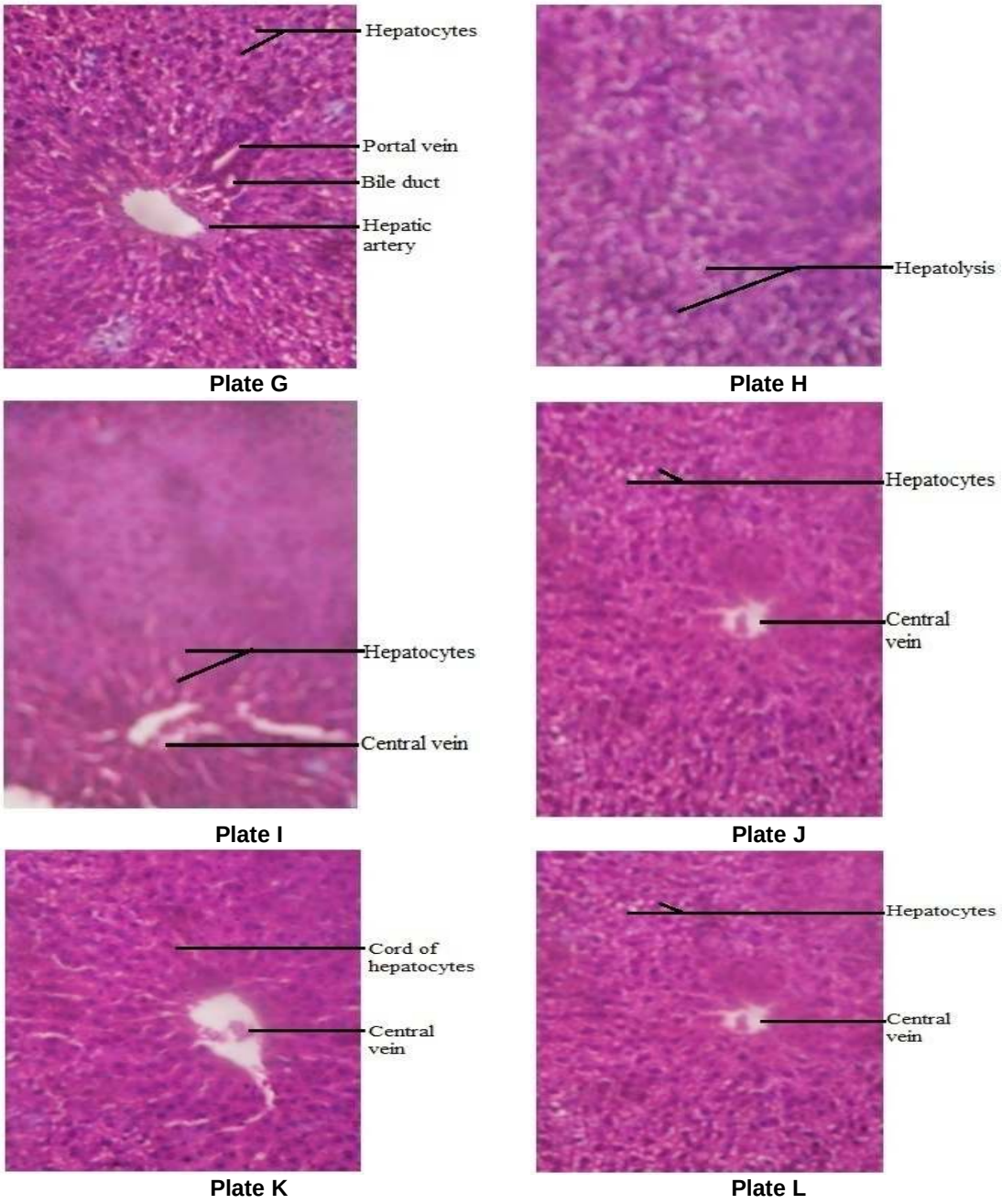

Plate G-L. Histopathology examination of liver

4. CONCLUSION

The results obtained in this study indicated that the administration of copper, induced hepatotoxicity, renal toxicity, and histopathological abnormalities as shown in the levels of SOD, GSH, GPx, catalase and elevated levels of MDA. Elevated levels of AST, ALP, ALT and bilirubin, and reduced levels of albumin, and total proteins affirmed the induced toxicity. Elevated levels of urea and creatinine and decrease in concentration of serum electrolytes indicated disruption in renal functionality on treatment with copper. Oral administration of 500 mg/kg b.w methanol extract of *T. occidentalis* had the most restorative effect among other treatments on these biochemical parameters. This suggests that *T. occidentalis* could

ameliorate the antioxidant status in copper-induced oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aruoma OI. Free radicals oxidative stress and antioxidants in human health. J. Am. Oil Chem. Soc. 1998;75:199-212.
2. Eseyin OA, Munavvar AS, Hassaan AR. A Review of the pharmacological and biological activities of the aerial parts of *Telfairia occidentalis* Hookf (Cucurbitaceae). Tropical Journal of Pharmaceutical Research. 2014;13(10): 1761-1769.
3. Johnson IT. Antioxidants and Antitumour Properties In: Antioxidants in Food Pokorny J Yanishlieva N and Gordon M (Eds) Cambridge: Woodhead Publishing Ltd. 2001;100-123.
4. Virgili F, Scaccini C, Packer L, Rimbach G. Cardiovascular disease and nutritional phenolics. In: Antioxidants in food Pokorny Yanishlieva J N and Rimbach G (Eds) Cambridge: Woodhead Publishing Ltd. 2001;87-99.
5. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea* Lincensed. Biomed Central Ltd.; 2008.
6. Wei Z, Shiow YW. Antioxidant activity and phenol compounds in selected herbs. J Agric Food Chem. 2001;49:5165-5170.
7. Oboh G, Akindahunsi AA. Change in the ascorbic acid total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutr Health. 2004;18:29-36.
8. Iweala EEJ, Obidoa O. Some biochemical haematological and histological responses to a long term consumption of *Telfairia occidentalis*- Supplemented Diet in Rats. Pak J Nutr. 2009;8:1199–1203.
9. Kayode OT, Kayode AA, Adetola AA. Therapeutic effect of *Telfairia occidentalis* on protein energy malnutrition- Induced liver damage. Res J Med Plant. 2009;3:80-92.
10. Kayode AA, Kayode OT, Adetola AA. *Telfairia occidentalis* ameliorates oxidative brain damage in malnourished rat. Int J Biol Chem. 2010;4:10-18.
11. Oboh G. Hepatoprotective property of ethanolic and aqueous extracts of *Telfairia occidentalis* (Fluted Pumpkin) leaves against garlic-induced oxidative stress. J Med Food. 2005;8:560-563.
12. Hamzah RU, Jigam AA, Makun HA, Egwim EC. Antioxidant properties of selected african vegetables, fruits and mushrooms: A review, mycotoxin and food safety in developing countries, Dr. Hussaini Makun (Ed.), InTech; 2013.
DOI: 10.5772/52771.
Available:http://www.intechopen.com/books/mycotoxin-and-food-safety-in-developing-countries/antioxidant-properties-of-selected-african-vegetables-fruits-and-mushrooms-a-review
13. Nwanna EE, Oboh G. Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. Pakistan Journal of Biological Sciences. 2007;10(16):2682–2687.
14. National institute of environmental health sciences-NIEHS NIH Publication no 85-23 “Respect for life”; 1985.
Available: http://www niehsnihgov/
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;95:351-358.
16. Aebi H. Catalase in vitro methods in enzymology. 1984;105:121-126.
17. McCord JM, Fridovich I. Superoxide dismutase An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244:6049–6053.
18. Sedlak J, Lindsay RH. Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman’s Reagent.

Analytical Biochemistry. 1968;25:1192-1205.

19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine. 1967;70:158-169.
20. Bartels H, Böhmer M. Micro-determination of creatinine. Clin Chim Acta. 1971;32:81-85.
21. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Annal Chem. 1967;39:971-974.
22. Doumas BT, Watson WA, Biggs HC. Albumin standards and Management of serum albumin with bromocresol green. Chin Chim Acta. 1971;31:87-96.
23. Flack CP, Woollen JW. Prevention of interference by dextran with biuret-type assay of serum proteins. Clin Chem. 1984;304:559-561.
24. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of clinical chemistry and molecular diagnosis 4th Ed Burtis Ashwood & Bruns Eds Elsevier Saunders. 2293; 2005.
25. Jendrassik L, Grof P. Colorimetric Method of Determination of bilirubin. Biochem Z. 1938;297:81-82.
26. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol. 1957;28:56-63.
27. Ali AS, Attalla FE. Phytoprotective and Antioxidant Effects of German Chamomile Extract against Dimpylate-Induced Hepato-Nephrotoxicity in rats. Advances in Life Science and Technology. 2014;19:33-41.
28. Moselhy SS, Husein KH. Hepatoprotective effect of Cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol Res. 2009;42:93-98.
29. Amel MS. Extract of *Coelatura aegyptiaca* a freshwater clam ameliorates hepatic oxidative stress induced by monosodium glutamate in rats. African Journal of Pharmacy and Pharmacology. 2011;5:(3): 398-408.
30. Omotayo MA, Ogundare OC, Longe AO. Hepatoprotective effect of *Mangifera indica* stem bark extracts on paracetamol-induced oxidative stress in albino rats. European Scientific Journal. 2015;11:24-299.
31. Dervis O, Resat O, Zafer G, Hafize U, Seval A. Copper-mediated oxidative stress in rat liver. Biological Trace Element Research. 2003;96.
32. Damodara RV, Saayi KG, Padmavathi P, Varadacharyulu N. Effect of *Emblica officinalis* against alcohol-induced biochemical changes in plasma and red blood cells of rats. African Journal of Biochemistry Research. 2007;1(6):101-105.
33. Padma VV, Baskaran R, Divyaa S, Priyaa LB, Saranya S. Modulatory effect of *Tinospora cordifolia* extract on Cd-induced oxidative stress in Wistar rats. Translational Integr Med Res. 2016;5(1): 48-55.
34. Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK. Protection against adriamycin-induced cardiotoxicity by garlic: Role of endogenous antioxidants and inhibition of TNF-a expression. BMC Pharmacol. 2003;3:1–9.
35. Akande IS, Akande BE, Gbenle GO. Toxicological and antioxidant effects of ethanolic extract of *Baphia nitida* on diazepam induced oxidative stress in rats. African Journal of Biochemistry Research. 2011;5(8):255-263.
36. Khan RA, Khan MR, Sahreen S, Shah NA, Khan AM. Effect of various fractions of *Launaea procumbens* on antioxidant enzymes in rats liver: Oxidative stress induced by potassium bromate ($KBrO_3$). African Journal of Pharmacy and Pharmacology. 2012;6(8):512-515.
37. Sahreen S, Khan MR, Khan RA. Hepatoprotective effects of methanol extract of *Carissa opaca* leaves on CCl4-induced damage in rat. BMC Compl Alter Med. 2011;11:48.

    DOI: 101186/1472-6882-11-48.
38. Fuentealba R, Davis E. Mechanisms of tolerance in the copper-loaded rat liver. Exp Mol Pathol. 1993;59:71–84.
39. Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 1989;73:334-339.
40. Omoregie ES, Osagie AU. Antioxidant properties of methanolic extracts of some Nigerian plants on nutritionally-stressed rats. Nigerian Journal of Basic and Applied Science. 2012;20(1):7-20.

41. Stockham SL, Scott MA. Fundamentals of veterinary clinical pathology Ames Iowa State University Press. 2002;434–459.
42. Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut S, Irmak MK. Erdosteine prevents doxorubicin-induced cardiotoxicity in rats. Pharmacol Res. 2003;48(4):377–382.
43. Malami I, Musa MS, Alhasan AM, Dallatu MK, Abdullahi K. Hepatoprotective activity of stem bark extract of *Mangifera indica* L on carbon tetrachloride-induced hepatic injury in Wistar albino rats. Int J Pharm Sci Res. 2014;5(4):1240-1245.
44. Pachathundikandi SK, Varghese ET. Blood zinc protoporphyrin serum total protein and total cholesterol levels in automobile workshop workers in relation to lead toxicity: Our experience. Indian J Clin Biochem. 2006;21(2):114-117.
45. Iweala EEJ, Osundiya AO. Biochemical Haematological and Histological Effects of Dietary Supplementation with leaves of *Gnetum africanum* Welw on Paracetamol-induced Hepatotoxicity in rats. International Journal of Pharmacology. 2010;6(6):872-887.
46. Kanchana NA, Mohammed S. Hepatoprotective effect of *Plumbago zylanica* on paracetamol induced liver toxicity in rats. Int Journal of Pharmacy and Pharm Sci. 2011;3(1):151-154.
47. Sanjiv C. The liver book: A comprehensive guide to diagnosis treatment and recovery Atria Jimcafe Company. 2002;35-41.
48. Anosike CA, Ugwu UB, Nwakanma O. Effect of ethanol extract of *Pyrenacantha staudtii* leaves on carbontetrachloride induced hepatotoxicity in rats Biokemistri. 2008;20(1):17-22.
49. Sudha A, Srinivasan P, Manikandaselvi S, Thinagarbabu R. Protective effect and antioxidant role of *Achyranthus aspera* against ethanol-induced oxidative stress in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(3).
50. Ojelabi BA, Areola JO, Babalola OO. Roles of *Glyphaea brevis* (Spreng) extract in cadmium induced hepatocellular damage and oxidative stress in rabbit Journal of Biotechnology and Pharmaceutical Research 2012;3(7):123-128.
51. Yakubu N, Oboh G, Olalekan AA. Antioxidant and Hepatoprotective Properties of Tofu (Curdle Soymilk) against Acetaminophen-Induced Liver Damage in Rats. Biotechnology Research International. 2013;7. Article ID 230142. DOI:10.1155/2013/ 230142.
52. Ebenyi LN, Ibiam UA, Aja PM. Effects of *Alliums sativum* extract on paracetamol – induced hepatotoxicity in albino rats. International Research Journal of Biochemistry and Bioinformatics. 2012; 2(5):93-97.
53. Nwangwa EK. Dose-dependent attenuating effects of aqueous extract of *Carica papaya* Seed on Carbon tetrachloride-Induced renal toxicity in rats. Advances in Life Sciences. 2012;2(4):124-127.
54. Ali FU. Ominyi M, Nwankwo OVU, Ibiam UA, Ogbanshi ME. Comparative effects of ethanolic extract of *Gongronema latifolium* and *Piper guineense* on blood electrolytes in ethanol exposed wistar rats. Biochem Anal Biochem. 2015;4:3.
55. Grover JK, Vats V, Rathi SS, Dawar R. Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. J Ethnopharmacol. 2001;76:233–238.
56. Yousef MI, Saad AA, El-Shennawy LK. Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food and Chemical Toxicology. 2009;47:1176–1183.

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